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NUTRITIONAL REQUIREMENTS OF ENTEROTOXIGENIC STRAINS OF STAPHYLOCOCCUS AUREUS

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ABSTRACT

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Title of Report: NUTRITIONAL REQUIREMENTS OF ENTEROTOXIGENIC STRAINS OF STAPHYLOCOCCUS AUREUS

Principal Investigators: Fritz T. Sulzer and Howard A. Peters

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The nutritional requirements for growth of four enterotoxigenic, two suspected enterotoxigenic, and two known non-enterotoxigenic strains of Staphylococcus aureus were investigated.

In a synthetic medium containing glucose, ammonium sulfate, other inorganic salts, thiamine and nicotinic acid, the minimum amino acid requirement for all strains were arginine and cystine, or,in absence of ammonium ion, a combination of arginine, cystine and glycine. In glucose-free media, more amino acids were required,for some strains up to eight, viz. arginine, aspartic acid, cystine, glycine, valine, proline, histidine and phenylalanine. Good growth comparable to that in non-synthetic media was attained with these eight amino acids in presence of glucose.

Glucose concentrations in the range from 1 to 50 g per liter were optimal in a synthetic, six-amino-acid medium. Higher concentrations inhibited growth progressively, and above 300 g per liter, no growth was noticeable. Low glucose concentrations limited growth, but even 0.1 g per liter allowed some.

The pH range for good growth extended from 5.5 to 8.0. Complete inhibition was noted below 4.1 to 4.6 and above 8.5 to 9.5 depending on substrate and strain.

Generally, no essential differences in nutritional requirements were found between enterotoxigenic and non-enterotoxigenic strains.

KEY WORDS:
Staphylococcus aureus: Growth in synthetic media; effect of pH on growth; effect of glucose concentration on growth.
Nutrition: Nutritional requirements of Staphylococcus aureus.
Amino acids: Requirement for growth of Staphylococcus aureus.
Growth: Nutritional requirements of Staphylococcus aureus.
Food Poisoning: Nutritional requirements of enterotoxigenic strains of Staphylococcus aureus.

NUTRITIONAL REQUIREMENTS OF ENTEROTOXIGENIC STRAINS OF STAPHYLOCOCCUS AUREUS

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A. INTRODUCTION.

Staphylococcal enterotoxin is believed to be the most common cause of food poisoning in the United States. Only relatively few strains of Staphylococcus aureus are known to be capable of producing enterotoxin, and only in limited types of food. Largely, meat-and-milk-base foods appear to provide a suitable growth medium for the production of enterotoxin. (Dack, 1956).

Most of the studies on growth and enterotoxin production have been made with complex, non-synthetic substrates. A few limited studies were conducted with synthetic media containing chemically defined ingredients (Gladstone,1937; Surgalla, 1947). Little information is available regarding nutritional factors in enterotoxin production. The lack of effective assay techniques for enterotoxins has been limiting such investigations since bioassays with monkeys or kittens had been, until recently, the only method for detecting toxin. Now the refined gel-diffusion technique for immunological antigen-antibody tests has resulted in a more specific, quantitative assay for enterotoxin. More intensive studies of the nutritional requirements for enterotoxin production have therefore become feasible.

The use of the gel-diffusion technique for any large scale study is limited at present to enterotoxin B which is produced by strains 243 and

S-6. No practical assay method is yet available for enterotoxin A, produced by strains 196E and 230, due to the lack of availability of the specific antiserum. As a first step in the investigation of enterotoxin production, the authors had proposed to study the nutritional requirements of some strains of Staphylococcus aureus. Subsequently, the enterotoxin production of selected strains would be investigated in relation to their growth on synthetic substrates. This report deals with the first phase of the proposed sequence.

Emphasis has been put in this study on the amino acid requirements of Staphylococcus aureus in synthetic substrates. Minimum requirements as well as optimal composition of such substrates were looked for. The effects of glucose concentration and of pH on growth were also studied experimentally.

B. ORGANISMS.

For some of the preliminary studies, a coagulase-positive strain of Staphylococcus aureus, obtained from the Department of Bacteriology, University of North Carolina, was used. Most experiments were performed, however, with eight strains received from the Milk and Food Research Branch of the Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio. These strains are characterized in Table 1.

Table 1: Staphylococcus Aureus Strains Under Investigation

Strain Number	Characteristic
196E	Produces Enterotoxin B
S-6	Produces Enterotoxin A and B
230	Produces Enterotoxin A
243	Produces Enterotoxin B
2073-2	Suspected Enterotoxigenic (American)
2483/54	Suspected Enterotoxigenic (England)
305	Non-Enterotoxigenic
111	Non-Enterotoxigenic

Forty-eight subcultures of each of the four known enterotoxigenic strains and twenty-four subcultures of each of the other strains were made in Difco Tryptone Glucose Extract Agar butt tubes with cork closures. Each tube was examined for visible growth after 24 hours incubation at 37°C and then sealed with melted paraffin wax. The tubes were placed in a deep freeze for storage at -25°C until needed. This procedure has been used for preserving the stock cultures without repeated transfers, thus minimizing the possibility of physiological changes due to selective growth in subcultures.

C. PRELIMINARY INVESTIGATIONS.

1. Turbidity of cell suspensions.

The photometric determination of turbidity was selected as the routine method for estimating growth. Some orienting experiments were performed to relate turbidity readings to viable cell counts (such a relationship naturally cannot be precise and is applicable only under rather strict

limitations as to age, substrate and other growth conditions. For comparative purposes in growth experiments, turbidity readings are, however, most suitable.)

In one series of experiments, Staphylococcus aureus was grown in tryptic soy broth and in casamino-acid-yeast-extract medium at 37°C. At intervals, samples were withdrawn and the turbidity and the viable cell concentration determined. The latter was obtained by dilution and plating on Trypton Glucose Extract Agar according to Standard Methods (APHA, 1960). The turbidity was determined with a Klett-Summerson Colorimeter (Model 900-3) using a filter 54 and Klett tubes (14 mm outer diameter). The results of one such experiment are shown in Figure 1.

In another series of experiments, the cells were suspended in buffered dilution water and the turbidity and viable cell count of sequential dilutions determined. This second series of experiments yielded more consistent results than the first. It was found that a Klett reading of approximately 50 corresponded to 10^8 cells per ml, while a Klett reading of approximately 150 indicated a cell concentration of 10^9 organisms per ml.

2. Growth on solid media.

Work done by Gladstone(1937), Sugalla (1947), and others established that the nutritional requirements for growth of Staphylococcus aureus were: inorganic salts, thiamine, nicotinic acid and amino acids, beside an energy source (preferably a carbohydrate). Since the present investigation was mainly concerned with defining the amino acid requirements more closely, a screening technique was needed which allowed rapid testing of numerous nutrient combinations but required only small amounts of individual amino acids. The auxanographic method appeared suitable, a solid media technique

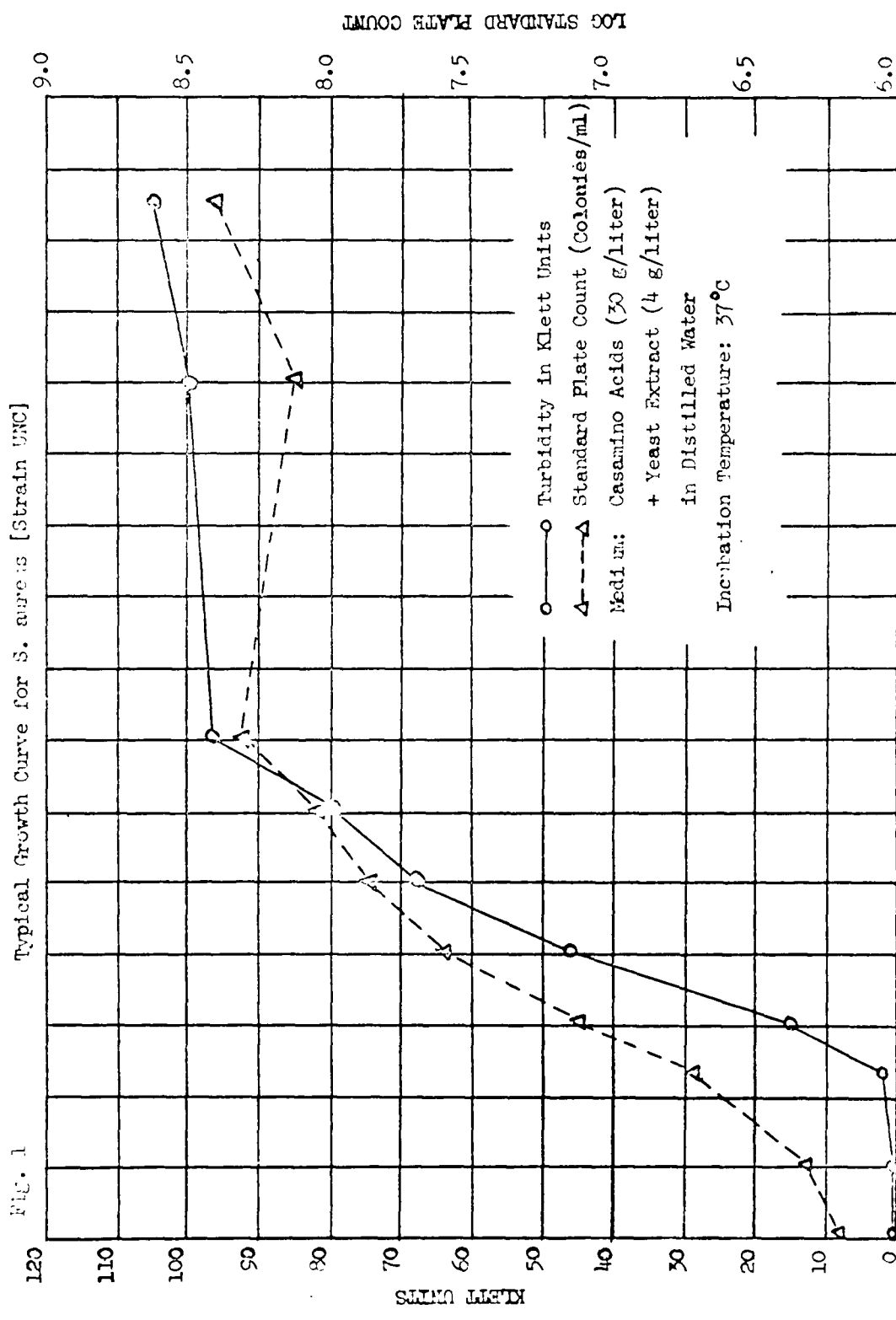
Typical Growth Curve for S. aureus [Strain UNC]
KLETT UNITS
LOG STANDARD PLATE COUNT
TIME IN HOURS
Turbidity in Klett Units
Standard Plate Count (Colonies/ml)
Medium: Casamino Acids (30 g/liter)
+ Yeast Extract (4 g/liter)
in Distilled Water
Incubation Temperature: 37°C

Fig. 1

in which test solutions are applied to localized areas and localized growth response is observed visually.

The general procedure was to inoculate minimal agar in Petri dishes with the staphylococcal strain under investigation and then spot different areas with known amino acid mixtures. Glucose was added in most cases with the amino acids. Growth was determined after 48 hours incubation at 37°C.

Two methods for inoculation were tried: Either a small amount of cell suspension was spread over the surface of the solidified minimal agar, or melted agar was inoculated with the cell suspension and then allowed to solidify.

Three methods for applying the test nutrients to the inoculated plates were tried:

a. Sterile absorbent paper discs (6 mm diameter) were placed on the agar surface and one drop of nutrient mixture added to each disc. Up to 7 mixtures (including controls) could be tested on one agar plate.

b. With a sharp edged sterile tool, small depressions were scooped out from the agar surface and a drop of nutrient solution added.

c. Sterile antibiotic test cups, made of stainless steel or porcelain, were pressed into the agar surface and a drop of nutrient mixture added to the cup.

The antibiotic cup technique in combination with liquid agar inoculation resulted in the best method. Uniform, clearly defined circular areas of growth were formed around the test cups containing adequate nutrients. The method did not permit quantitative growth studies, but was very useful for rapid screening of the numerous nutrient combinations. The amino acid

combinations, tested later in liquid cultures, were mainly selected on the basis of such qualitative screening tests on solid medium. Some typical qualitative results are given in Table 2.

Table 2: Growth Response of Four Enterotoxigenic Strains of Staphylococcus aureus to Amino Acids and Ammonium Salt on Solid Medium (auxanographic method)

Supplements to Basal Medium	Growth Response after 48 Hours (37° C.)			
	196E	S-6	230	243
none	-	-	-	-
glucose	-	-	-	-
ammonium + glucose	-	-	-	-
cystine + glucose	-	-	-	-
cystine + ammonium + glucose	-	-	-	-
arginine + glucose	-	-	-	-
arginine + ammonium + glucose	-	-	-	-
arginine+cystine+ammonium+glucose	+	+	+	+
glycine+cystine+ammonium+glucose	-	-	-	-
arginine+cystine+glycine+glucose	+	∓	+	+
casamino acids 3%	∓	∓	∓	∓
casamino acids + glucose	+	+	+	+
tryptic soy broth (as control)	++	+	++	++

Basal medium:
Inorganic salts and vitamins as listed in Table 3, agar: 1%

Growth response:
- - no growth
- ∓ slight, dubious growth
- + fair growth
- ++ good growth

Occasional inconsistencies between growth on agar and growth in liquid cultures with various amino acid combinations were noticed. Impurities in the ordinary commerical grades of granular agar used possibly influenced the results. Furthermore, it was found that glucose, added as an energy source in most tests, had to be sterilized separately from the other nutrients to obtain reproduceable results.

3. Growth in liquid media.

Originally ordinary culture tubes (16 mm x 150 mm, with metal caps) with 10-ml substrate portions were used for growth experiments. They proved unsatisfactory, since growth was not well suspended, but tended to accumulate in a layer at the bottom of the tubes. Continuous shaking during incubation did not lead to better dispersion of growth. All subsequent experiments were therefore carried out in erlenmeyer or similar flasks providing a wide bottom area.

D. GROWTH STUDIES WITH SYNTHETIC SUBSTRATES.

The following procedures were used in all subsequent experiments:

A basal salts-vitamin solution (see below) was prepared in liter amounts, sterilized by autoclaving and stored at 4°C until used. Similarly, sterile glucose solutions (500 g per liter) were prepared in 200-ml amounts. Amino acids and ammonium salt were indivudally weighed and dissolved in portions of the basal salts-vitamin solution which was then resterilized by autoclaving. If required, sterile glucose solution was added to the cooled amino acid-salts solution. The pH of the mixture was then adjusted to approximately 7.0 with sterile 1 N sodium hydroxide. The finished substrate was pipetted into previously capped and sterilized flasks, using sterile techniques at all times. As culture flasks 125-ml erlenmeyers,

capped with aluminum foil, or special 300-ml erlenmeyer flasks with Klett tubes fused into the side and fitted with stainless steel caps were used. The latter received 50 ml medium, while 25 ml were pipetted into the former.

The inoculum was prepared from 24-hour tryptone glucose agar slants of the stock cultures. The bacterial cells were washed from the surface of the slants with sterile, buffered water and pipetted into the culture flasks to give a concentration of approximately 5×10^6 organisms per ml medium. The flasks were then rotated to distribute the inoculum and incubated at 37°C without further agitation. Immediately before sampling, the flasks were shaken vigorously.

Routinely, growth was determined by turbidity measurements with a Klett colorimeter (filter 54, tubes with 14 mm diameter), as described before. All turbidity values given are corrected, i.e. blank readings of the uninoculated substrates have been subtracted from the actual readings of the inoculated cultures. Usually samples were taken 24 and 48 hours after inoculation.

The basic stock salts-vitamin solution consisted of the components listed in Table 3.

Table 3: Composition of the Basal Salts-Vitamin Solution

KH_2PO_4	2.0 g per liter
$MgSO_4 \cdot 7\ H_2O$	0.1 g per liter
$FeSO_4 \cdot 7\ H_2O$	0.01 g per liter
Nicotinic acid · HCl	1.2 mg per liter
Thiamine · HCl	0.04 mg per liter
in distilled water	

Adapted from Surgalla (1947)

Cystine was always added in amounts corresponding to 0.1 g per liter (0.01%), i.e. approximately at saturation concentration. All other amino acids were added in the individual concentration of 1.0 g per liter (0.1%), unless otherwise stated. Ammonium sulfate was similarly added at a 1.0 g per liter level. Glucose was usually supplied at a 10 g per liter concentration.

1. Effect of various amino acid mixtures.

Approximately fifty different combinations of the twelve amino acids (listed in Table 4) were tested, partly in conjunction with ammonium salt, for their effect on growth.

Table 4: Amino Acids Tested in Growth Experiments

L - arginine	L - leucine
DL - aspartic acid	DL - methionine
L - cystine	DL - phenylalanine
glycine	L - proline
L - histidine	DL - serine
DL - isoleucine	DL - valine

Some of the amino acid combinations were tested only with the four enterotoxigenic strains. Certain mixtures were tested several times. Table 5 illustrates the growth response obtained with some selected synthetic substrates and a casein hydrolysate substrate.

Table 5: Growth Response of Three Enterotoxigenic Strains to Selected Amino Acid Mixtures

SUBSTRATE		TURBIDITY IN KLETT UNITS					
*Ref. No.	Supplements to Basal Medium	196E		S-6		243	
		24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.
2a	cys + NH_4 + Glu	0	4	0	3	0	0
3a	arg + cys + Glu	4	4	3	3	3	3
4a	arg + gly + Glu	4	5	0	1	2	2
1	arg + cys + NH_4+ Glu	9	51	10	56	6	65
3	arg + cys + gly + Glu	30	61	24	13	39	61
13	6 amino acid + Glu	33	45	25	47	24	38
25	8 amino acid + Glu	85	110	60	368	74	338
32	12 amino acid + Glu	58	72	84	118	77	112
35	casamino acid + Glu	173	238	206	264	190	280
26	8 amino acid 0.1% each (without Glu)	17	40	12	30	17	40
28	8 amino acid 0.4% each (without Glu)	4	31	4	9	4	28
34	casamino acid 3 % (without Glu)	70	77	37	47	30	47

Abbreviations: cys = cystine gly = glycine Glu = glucose
arg = arginine NH_4 = ammonium salt

*Ref. No. refers to substrate number in appendix

The detailed data of these experiments with amino acids are listed in the Appendix. The results can be summarized as follows:

The minimum amino acid mixture on which all eight strains grew within 48 hours at a glucose concentration of 10 g per liter consisted of arginine, cystine and glycine. Growth also occurred in a mixture of arginine, cystine and ammonium sulfate with 10 g per liter of glucose.

Cystine could be replaced by methionine in a glucose containing medium only in presence of valine, arginine and glycine.

In glucose-free media, there were differences in the combinations of amino acids required by the individual strains for growth. Most strains grew in a mixture of the eight amino acids arginine, aspartic acid, cystine, glycine, valine, proline, histidine and phenylalanine. When supplemented with 10 g per liter glucose, this mixture proved to be a very good growth medium.

If the concentrations of the individual amino acids, with the exception of cystine, were increased from 1 g per liter to 3, 4, or 5 g per liter in an attempt to substitute amino acids for glucose as carbon and energy source, variations among strains were found. Some strains were able to grow in a mixture of arginine and glycine at 5 g per liter each, in presence of cystine (at 0.1 g per liter).

No growth occurred in mixtures composed of amino acids, vitamins and glucose, but lacking inorganic salts. Increasing the number of amino acids (or their concentration) led in several cases to inhibitory effects.

There were no essential differences in nutritional requirements for growth between the enterotoxigenic and the non-enterotoxigenic strains taken collectively.

2. Effect of glucose concentration on growth

In a series of experiments, the effect of glucose concentration on the growth of strains 196E and S-6 was determined.

The experimental procedures described on page 8 f. were used. The medium consisted of the basal salts-vitamins solution (see table 2) and the six amino acids arginine, aspartic acid, cystine, glycine, proline and valine (Substrate No. 13). The glucose concentration was varied from 0.1 to 300 g per liter. The finished substrates were distributed in 50-ml portions into 300-ml erlenmeyer flasks, inoculated with either strain and incubated at 37°C. Samples were taken after 24 and 48 hours.

At a glucose concentration of 300 g per liter, no growth was noticeable for either strain. At 250 g per liter, both strains grew within 48 hours, although growth was not visible after 24 hours. At somewhat lower glucose concentrations, strain S-6 appeared retarded after 24 hours, but little difference between the two strains was noticeable after 48 hours. Growth of both strains became increasingly better as the glucose concentration was lowered. Optimum growth in both strains occurred at concentrations between 1 and 40 g per liter. There was little difference in growth at various concentration levels within these limits. As the glucose concentration was lowered below 1 g per liter, growth decreased, but there was still significant growth at 0.1 g per liter.

In all cases where growth occurred, higher turbidity values were obtained with strain 196E than with strain S-6.

3. Effect of pH on growth.

One non-synthetic and two synthetic media were used in determining the upper and lower pH limits and the optimum pH for growth. Difco

Tryptic Soy Broth was the non-synthetic medium, while the two others consisted of basal salts-vitamin solution (described previously)in combination with amino acids and 10 g per liter glucose. One of these substrates (No. 13, Appendix) contained six amino acids, viz. arginine, aspartic acid, cystine, glycine, valine and proline (at 1.0 g per liter, cystine at 0.1 g per liter), the other (No. 25, Appendix) had eight, viz. histidine and phenylalanine in addition to the above six. The pH was adjusted in the culture flasks to various levels between 3.80 and 10.70 with .1 N sodium hydroxide and .1 N HCl respectively before inoculation. The strains 196E and S-6 were tested. Incubation temperature was 37°C. Cell density and pH were checked periodically (12, 24, 48, 72, and 96 hours after inoculation). The pH determinations were made with a Beckman Zeromatic pH meter connected to a glass-calomel electrode pair. A Klett colorimeter served for cell density measurements.

The results of these experiments are summarized in Table 6. It can be seen from these data that a pH of approximately 7.0 permitted optimal growth of both strains in any of the three media tested. The non-inhibitory pH range for both strains was greater in Tryptic Soy Broth than in either of the two synthetic media. Generally, fairly good growth occurred within the relatively broad pH range from 5.0 to 8.5.

Table 6: Effect of pH on Growth of Strains 196E and S-6 in Three Media

Strain	Relative Growth (after 72 hours incubation)	pH Range		
		Tryptic Soy Broth	Six-amino-acid medium	Eight-amino acid medium
196E	optimal	6.5-8.5	6.0-7.3	6.4-7.9
	good	5.5-9.7	5.5-7.7	5.2-8.7
	poor	$\leq$ 3.8 $>$ 9.9	$\leq$ 4.6 $>$ 8.6	$\leq$ 4.6 $>$ 9.0
S-6	optimal	5.2-8.2	6.5-7.3	6.3-7.7
	good	4.5-9.7	5.7-7.7	5.2-8.0
	poor	$\leq$ 3.7 $>$ 9.9	$\leq$ 4.3 $>$ 8.4	$\leq$ 4.6 $>$ 8.3

E. STUDIES IN PROGRESS.

In July 1963, a consultation visit was held with Dr. Merlin S. Bergdoll at the Food Research Laboratories of the University of Chicago. As a result of this visit, it was decided to limit the studies on the production of toxin to the B enterotoxin (produced by strains S-6 and 243), due to the lack of availability of a purified A enterotoxin and its specific antiserum. A supply of purified B enterotoxin and specific antiserum was obtained from Dr. Bergdoll. Methods, equipment and reagents for the enterotoxin production study were discussed in detail during the visit. The enterotoxin studies will be started in October 1963. Purification by ion exchange chromatography of the enterotoxin produced in liquid media will not be necessary for the gel-diffusion assay method used. Concentration by dialysis or dilution in buffer solution will be employed to obtain the proper concentration of enterotoxin.

At present, investigations are under way concerning the effect of varying glucose concentrations in aerobic and anaerobic cultures and the influence of carbon dioxide on aerated cultures.

F. REFERENCES.

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G. ACKNOWLEDGEMENTS.

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A P P E N D I X

Table

of selected liquid substrates

and growth responses obtained

with eight strains of Staphylococcus aureus

Substrate reference number and composition of each substrate (i.e. supplements added to basal salts-vitamin solution)are stated.

Growth response is expressed in corrected Klett units (actual reading at 24 and 48 hours minus reading of uninoculated medium).

Repeated testing of a substrate is indicated by two or more Klett readings listed with a substrate under any strain number and sampling time.

Growth of Eight Strains of Staphylococcus in Liquid Medium Cultures

No.	Medium Composition	196E 24hr	196E 48hr	S-6 24hr	S-6 48hr	230 24hr	230 48hr	243 24hr	243 48hr	2073-2 24hr	2073-2 48hr	2483/54 24hr	2483/54 48hr	305 24hr	305 48hr	111 24hr	111 48hr
1.	arginine cystine $(NH_4)_2$ SO_4 glucose	9 11 19	51 57 58	10 13 18	56 70 59	8 19 27	63 74 59	6 13 10	65 46 81	7 7	7 10	24 38	63 58	7 3	7 66	16 18	69 40
2.	arginine 0.5% cystine $(NH_4)_2$ SO_4 0.5%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.	arginine cystine glycine glucose	30 23	61 49	24 24	13 9	36 38	76 68	39 22	61 22	0 6	7 5	28 31	56 50	3 8	9 7	32 35	68 54
4.	arginine 0.5% cystine glycine 0.5%	4	5	4	7	5	12	6	12	4	9	4	9	6	13	7	13
5.	arginine aspartic acid cystine glycine glucose	18 19	43 50	25 12	69 58	31 27	57 59	21 11	54 19	3	5	26	61	6	24	26	61
6.	arginine cystine glycine proline glucose	21 19	40 52	21 19	12 25	31 17	57 77	34 24	74 79	2	6	17	51	24	69	17	57

Continued

Continued

Growth of Eight Strains of Staphylococcus in Liquid Medium Cultures

Medium		196E		S-6		230		243		2073-2		2483/54		305		111	
No.	Composition	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
7.	arginine cystine glycine valine glucose	22 18	52 61	22 6	65 64	33 17	66 79	21 9	87 65	2	7	10	55	10	18	15	80
8.	arginine glycine methionine valine glucose	5	24	0	10	8	30	0	11	1	5	0	23	0	3	0	29
9.	arginine aspartic acid cystine glycine proline glucose	5 19	22 41	12 20	36 66	22 27	39 63	0 26	9 64	0	8	27	55	14	68	28	67
10.	arginine aspartic acid cystine glycine valine glucose	5 16	29 47	3 11	29 45	11 11	33 40	0 13	17 35	4	9	14	37.	7	51	13	43
11.	arginine aspartic acid cystine proline valine glucose	0 2	0 30	0 2	14 26	0 2	14 24	0 2	0 8	2	7	2	29	2	6	2	30

Continued

Continued

Growth of Eight Strains of Staphylococcus in Liquid Medium Cultures

Medium		196E		S-6		230		243		2073-2		2483/54		305		111	
No.	Composition	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	43hr	24hr	48hr	24hr	48hr	24hr	48hr
12.	arginine aspartic acid cystine glycine $(NH_4)_2$ SO_4	4	7	4	4	4	7	4	4	4	4	4	4	4	4	4	15
13.	arginine aspartic acid cystine glycine valine proline glucose	33 10	45 42	25 8	47 14	38 22	51 50	24 3	38 4	0	0	16	34	7	11	23	47
14.	arginine 0.3% aspartic acid 0.3% cystine glycine 0.3% valine 0.3% proline 0.3%	5	5	5	5	8	20	7	14	5	13	5	5	13	38	9	23
15.	arginine 0.5% aspartic acid 0.5% cystine glycine 0.5% valine 0.5% proline 0.5%	3	3	4	7	10	19	15	31	16	25	8	7	15	38	13	14

Continued

Continued

Growth of Eight Strains of Staphylococcus in Liquid Medium Cultures

Medium		196E		S-6		230		243		2073-2		2483/54		305		111	
No.	Composition	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
16.	arginine aspartic acid cystine glycine proline $(NH_4)_2\ SO_4$	7	8	7	8	15	33	7	20	7	8	7	18	9	35	13	20
17.	arginine aspartic acid cystine glycine valine $(NH_4)_2\ SO_4$	7	6	5	6	14	42	6	8	9	13	5	7	10	33	10	30
18.	arginine aspartic acid cystine histidine glycine proline valine	0	0	0	0	1	7	0	0	1	7	0	0	0	0	1	3
19.	arginine aspartic acid cystine glycine leucine proline valine	0	0	0	0	1	9	0	0	1	10	1	9	1	4	0	0

Continued

Continued

Growth of Eight Strains of Staphylococcus in Liquid Medium Cultures

Medium		196E		S-6		230		243		2073-2		2483/54		305		111	
No.	Composition	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
20.	20. arginine aspartic acid cystine glycine proline valine $(NH_4)_2 SO_4$	7	7	7	12	17	55	7	25	11	15	7	13	13	45	17	53
21.	21. arginine aspartic acid cystine glycine isoleucine leucine serine valine	0	58	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22.	22. arginine aspartic acid cystine glycine leucine phenylalanine proline valine	8	17	6	12	20	54	10	16								
23.	23. arginine aspartic acid cystine glycine histidine proline valine $(NH_4)_2 SO_4$	3	4	1	11	10	44	1	19	5	31	1	4	5	37	4	32

Continued

Growth of Eight Strains of Staphylococcus in Liquid Medium Cultures

No.	Medium Composition	196E		S-6		230		243		2073-2		2483/54		305		111	
		24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
24.	arginine aspartic acid cystine glycine phenylalanine proline valine $(NH_4)_2\ SO_4$	4	20	2	6	2	24	12	53	4	11	5	7	5	44	9	40
25.	arginine aspartic acid cystine glycine histidine phenylalanine proline valine glucose	85	110	60	363	85	403	74	338	98	156	76	358	81	178	93	323
26.	arginine aspartic acid cystine glycine histidine phenylalanine proline valine	17 2	40 21	12 2	30 6	33 12	75 55	17 3	40 26	26 4	63 34	10 3	27 6	18 3	57 31	23 3	54 15

Continued

Growth of Eight Strains of Staphylococcus in Liquid Medium Cultures

Composition	196E		S-6		230		243		2073-2		2483/54		305		111	
	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
[illegible] 0.4%	7	173	12	131	7	155	51	131	63	163	49	135	60	162	73	150
[illegible] 0.4%	3	91	4	9	10	70	4	23	10	43	4	8	4	46	10	40
[illegible]ine	15	50	24	40	12	43	57	50								

Continued

Continued

Growth of Eight Strains of Staphylococcus in Liquid Medium Cultures

	Medium	196E		S-6		230		243		2073-2		2483/54		305		111	
	Composition	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
30.	[illegible] acid [illegible] [illegible] [illegible] [illegible] [illegible] proline [illegible] [illegible]	[illegible]	7	[illegible]	7	[illegible]	7	[illegible]	15								
31.	[illegible] [illegible] acid [illegible] [illegible] [illegible] [illegible] [illegible] proline [illegible]	17	[illegible]	15	22	[illegible]	57	21	26								
32.	[illegible] [illegible] acid [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible]	[illegible]	72	[illegible]	115	70	105	77	112								
		[illegible]	[illegible]	75	125	62	121	63	108	63	141	87	102	85	147	60	82

Growth of Eight Strains of Staphylococcus in Liquid Medium Cultures

	Strain	195E		S-6		230		243		2073-2		2483/54		305		111	
	Composition	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
33.	[illegible] acid [illegible]ine [illegible]ine [illegible]line [illegible]oleucine [illegible]ine [illegible]thionine [illegible]phalanine [illegible]line [illegible]ine [illegible]ine	33	50	5	27	7	50	30	35	3	21	4	21	8	43	11	48
[illegible].	Casamino Acids (Bacto)	70	77	37	47	83	78	30	47								
3[illegible].	Casamino Acids (Bacto) glucose	173	238	206	264	170	240	190	280								

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